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Cardiovascular Effects of Losartan and Its Relevant Clinical Application

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Abstract

The renin-angiotensin system (RAS) plays an important role in the homeostasis of the cardiovascular system and in the development of cardiovascular diseases. An abnormal expression or over activation of the local RAS in the heart and vasculature system is one of the most common mechanisms in pathophysiological processes in cardiovascular diseases. This also provides a basis for medical prevention and treatments using chemical approaches. Losartan is a selective nonpeptide antagonist against type 1 angiotensin II receptors (AT1R), and has been applied in medical treatments of a variety of cardiovascular diseases, including essential hypertension. This article reviews direct and indirect cardiovascular effects of losartan on the heart and blood vessels. It summarizes the chemical basis of AT1R for the action site of losartan, focuses on the mechanisms underlying the action of losartan involved in both the heart and vasculature, and reviews the information that may be helpful in the development of new chemical candidates or approaches in the war against cardiovascular diseases.

Keywords

Angiotensin II; heart; losartan; renin-angiotensin system; cardiac remodeling; hypertension

1. INTRODUCTION

In the systemic renin-angiotensin system (RAS), the substrate angiotensinogen is mainly synthesized and released from the liver. Once in the circulation, it is cleaved by rennin secreted from the juxtaglomerular apparatus in the kidney. The product of decapeptide angiotensin (Ang) I is further converted into the octapeptide Ang II by a membrane-bound metalloprotease named angiotensin converting enzyme (ACE) that is in a high density on the surface of pulmonary vascular endothelium. Ang II is the most active peptide of the RAS and binds to Ang II subtype 1 (AT1) and subtype 2 (AT2) receptors for its actions in physiological and/or pathophysiological processes [1,2].

In addition to the systemic RAS, the local RAS with its key components including angiotensinogen, renin, ACE, and Ang II, has been demonstrated in almost all major organs.

Among them, the RAS in the cardiovascular system has attracted a great attention in its physiological and pathophysiological functions [3]. The existence of the cardiac RAS was first suggested by the discovery of renin and the renin-like activity (or angiotensinogen) in the heart [4]. Since these initial discoveries, most intrinsic components of the RAS including angiotensinogen, angiotensins, converting enzymes, as well as AT1 and AT2 receptors have been demonstrated in the heart and vasculatures [2].

Studies over the last decades have shown that the local RAS and its receptors play an important role in the control of structural and functional remodeling in the heart and vasculature, as well as in the development of cardiovascular diseases [5]. On the other hand, medicational studies and clinical trials have focused on the prevention and treatments of cardiovascular diseases with Ang II receptor antagonists [6]. Among them, the selective AT1 receptor antagonist losartan received a great attention in a number of experimental and medical studies [7]. It is clear that the RAS and AT1 receptors play a critical role in the regulation of renal functions [8,9], and this has been reviewed recently [10]. The present article focuses on the local RAS in the heart and vasculature and reviews the molecular and cellular mechanisms in cardiovascular disease development *via* AT1 receptors, as well as the application of losartan in the prevention and treatments. Given the importance of the local RAS in the regulation of cardiovascular homeostasis, the information on the relationship between the tissue RAS in normal and abnormal conditions and the AT1 receptor blockers should shed light on the mechanisms for the treatments of cardiovascular diseases.

2. LOCAL RAS IN THE CARDIOVASCULAR SYSTEM

2.1. Cardiac Local RAS

Clinical and experimental evidence indicates that Ang II has pluripotential effects on the cardiac tissue under pathophysiological conditions. Although the RAS components in the circulation play an important role in the process, recent studies have demonstrated that local angiotensins in the heart may be more potent in contributing to cardiac tissue pathogenesis.

Angiotensinogen mRNA in the heart has been detected in various species [4,11–13]. Angiotensinogen can be synthesized in the cardiomyocyte and fibroblast *de novo* [14,15]. However, the majority of angiotensinogen in the heart is from the myocardial interstitium whose source is originated from the circulation [16].

A large body of evidence shows that renin mRNA in the myocardium (cardiomyocytes and fibroblasts) is not identical to that in the juxtaglomerular cell, and the cardiac protein product is a truncated form lacking the prefragment of prorenin as that from juxtaglomerular cells [17]. Whether the full-length renin mRNA exists in the heart is still controversial [4,13,18–21]. However, the presence of local renin protein in the heart has been confirmed [22–25]. Renin in the heart also may come from the circulation and diffuse into the interstitium [26].

Angiotensin converting enzyme (ACE) mRNA [13,27] and its enzymatic activity [28,29] have been demonstrated in the heart. Cardiac blood vessels and the endocardium are the predominant cellular parts for the ACE expression [28,30]. In addition, ACE also has been found in cardiomyocytes and fibroblasts [31–33]. In addition to ACE, ACE2, a captopril insensitive homologue of ACE, is expressed and functional in the heart [34–36]. ACE2 expression has been demonstrated not only at the endothelial and smooth muscle cells in intramyocardial vessels, but also found at the cardiomyocytes and interstitial fibroblasts [37,38]. Additionally, a chymotrypsin-like serine protease-chymase presents a potent alternative pathway of producing Ang II in the heart [39,40]. Unlike ACE and ACE2, the

chymase was found in the mast cells in the heart, in which it stored as a macromolecular complex with heparin proteoglycan in the cytoplasmic secretory granules [41,42].

Therefore, Ang II can be produced in the heart by one of the following ways: a) it can be synthesized *in situ* in the heart independent of the circulation [43–45], b) the precursor substrates, enzymes, and Ang II itself can all be sequestered from the circulation [46]. Various lines of evidence show that AT1 and AT2 receptors are expressed in cardiomyocytes [47–49]. In addition, fibroblasts also express AT1 receptors [50,51], as well as AT2 receptors in the heart under several conditions [52–54]. Furthermore, all the components of the RAS exist in the coronary vasculature, and this will be discussed in detail below.

2.2. Vascular Local RAS

Angiotensinogen mRNA is expressed abundantly in periadventitial adipocytes in the vasculature [55]. Notably, angiotensinogen in blood vessels is mainly attributed to diffusion from adventitial fat cells [56,57]. Like in the heart, renin in the vasculature can be either synthesized *in situ* [58] or derived from the circulation mediated by prorenin/renin acting sites [59] and/or by M6P receptors [60] predominantly located on endothelial cells. Both protein and mRNA of ACE are detected in the endothelium [61], smooth muscle cells [62], and adventitia layer of the vascular wall [63]. ACE2 is detected only in the endothelial and smooth muscle cells [64]. As a result, the local vascular Ang II levels can be regulated *in situ*. AT1, AT2, and Mas receptors all are expressed and located in endothelial cells [65–70].

3. CHEMISTRY OF AT1 RECEPTORS AND LOSARTAN

3.1. The Chemical Structure of AT1 Receptors

The AT1 receptor belongs to the family A (rhodopsinlike family) of G protein-coupled receptors (AGPCR) [47], and is composed of 359 amino acid residues of about 41kD in molecular weight. The AT1 receptor gene is located on the chromosome 3q21–q25 in human [71] (Fig. 1). In rodents, the gene loci for the AT1a and AT1b subtypes are on the chromosome 17q12, 2q24, and chromosome 13, 3, respectively [47,72]. By using the hydropathy analysis or multiple sequence alignment method [47,73], the AT1 receptor has been widely ascertained to be a seven transmembrane protein, of which each transmembrane segment is a hydrophobic α helix buried in the lipid membrane. Furthermore, using a resolved rhodopsin as a template [74], the gross tertiary structure of the AT1 receptor is modeled as a seven-transmembrane bundle, intercalated by three extracellular and intracellular loops, together with the N-terminus and C-terminus in the extracellular and intracellular regions, respectively [75]. In addition, there are two disulfide bridges in the extracellular domain of the AT1 receptor. The conserved one is between the first and second extracellular loop, and another pair is located at the N-terminal region and the third extracellular loop for maintaining proper conformation of the receptor [76]. Posttranslational modification of the AT1 receptor has been demonstrated. Three asparagine residues are constitutively glycosylated, with one in the N-terminal region and the other two in the second extracellular loop [77]. As a result, the molecular weight of the native intact AT1 receptor is about 65kDa, but not 41kDa as described above. In the intracellular domains, this receptor consists of three intracellular loops, C-terminal tail, and several consensus residues for phosphorylation by kinases such as protein kinase C, serine/threonine kinase, GPCR kinase, and protein tyrosine kinase [47].

3.2. Chemical Interaction Between the AT1 Receptor and Losartan

The inhibition of the AT1 receptor by losartan is surmountable, which could be reversed by Ang II following a competitive mechanism [78]. After binding to the positions in the

extracellular halves of the fifth (Thr198) and sixth helices (Ser253), losartan can be extended along the central cavity of the seven transmembrane bundle of the AT1 receptor among the helices, possibly involves Asn111 in the third helix, Asn294 and Asn295 in the seventh helix, and can achieve the maximum limits of the cytoplasmic interface of the receptor, which may contact to Leu300 and Phe301 in the seventh helix [79–81] (Fig. 2).

3.3. Brief Overview of Pharmacological Properties of Losartan

The pA_2 (-Log of the dissociation constant of receptor-antagonist complex) of losartan is around 10 [82,83]. After oral uptake, losartan undergoes either oxidative or conjugative metabolism in the liver, and then is transformed to hydroxylated and carboxylic acid derivatives (EXP3174), or glucuronide metabolite with modification in the tetrazole moiety [83]. In clinic, losartan has been often used for the treatment of cardiovascular disorders (e.g. hypertension, heart failure, cardiac infarction, etc.), and for controlling of the progression of a variety array of renal diseases, including diabetic nephropathy, type 2 diabetes, nephritic syndromes, and glomerulonephritis. It should be noted that doses of losartan in clinical applications are diverse in various diseases treated. The optimal dose range in the treatment of hypertension is 25–100 mg/d, although the dose of losartan could be elevated with at a minimum of 100 mg/d depending on treatment requirements and disease conditions [84–86].

4. LOSARTAN AND THE CARDIAC RAS

Clinically, the heart is affected by a variety of diseases that can be briefly summarized in three areas: 1) overload problems (pressure overload, e.g. hypertension; and volume overload, e.g. valvular heart diseases), 2) injury problems (e.g. myocardium infarction and diabetes), and 3) genetic problems (e.g. inherited cardiomyopathy). Alterations of the cardiac RAS have been indicated in all three pathological situations. Several lines of evidence show the elevation of angiotensinogen, renin, and ACE mRNA, as well as chymase activity in cardiac diseases [87–93]. This indicates that either *de novo* or *in situ* formation of Ang II is increased in the heart under pathophysiological conditions [94]. The elevated cardiac Ang II is caused by either an increased regulatory exocytosis locally in the heart or enhanced sequestration of major components of the RAS in the circulation. In addition, cardiac AT1 and AT2 receptor expression also could be altered under various pathological conditions [95–97].

It has been demonstrated that losartan blocks Ang II-induced upregulation of angiotensinogen, renin, and ACE mRNA [98]. As a result, the production of Ang II by *de novo* or *in situ* routes is attenuated or inhibited. In addition, losartan induces the up-regulation of myocardium ACE2 mRNA levels [99]. The elevated ACE2 would in turn catalyze Ang II to form Ang [1–7] that also plays a role in lowering the cardiac levels of Ang II. Furthermore, increased intracardiac Ang [1–7] may bind to Mas receptors to elicit several counteractive effects against AT1 receptor activation [100,101].

The sympathetic nerve transmitter noradrenalin in the heart can enhance the expression of angiotensinogen mRNA in both cardiomyocytes and fibroblasts *via* α_2 and/or β_1 receptors [2]. The blockade of AT1 receptors on the sympathetic nerve terminal by losartan reduces catecholamine release, which subsequently attenuates the expression of angiotensinogen transcription. Therefore, this may be another pathway for losartan to reduce Ang II concentrations in the myocardium. In addition, mechanical stimuli to the heart can cause both exocytosis of Ang II and angiotensinogen transcript expression [94]. The normalization of hemodynamics by losartan should inhibit this pathway and further reduce intracardiac Ang II levels.

4.1. The Potent Effects of Losartan on the Heart

Remodeling in the cardiovascular system or the heart has attracted a great attention in the studies of last two decades. The following sections review the beneficial effects of losartan in the management of the problems in cardiac and ventricular remodeling and hypertrophy.

4.1.1. Losartan and Cardiac Remodeling—Cardiac remodeling has been defined as genome expression. During the remodeling process, molecular, cellular, and interstitial changes are manifested as alternations in size, shape, and functions of the heart [102]. Losartan has been shown to have beneficial effects against pathological remodeling in the cardiovascular system [103] (Fig. 3).

4.1.2. Losartan and Ventricular Remodeling—Ventricular remodeling is a clinical process involved in the regulation of plasticity in ventricular size, shape, and function [104], resulting from various pathological conditions, including hypertension [105], myocardial infarction (MI) [106], heart valvular disease [107], heart failure [108], cardiomyopathy [109], and other conditions such as protracted bed rest, persisted application of ventricular assist device, and prolong space travel [110]. As a consequence, ventricular hypertrophy and electrical remodeling may occur. The potential benefits of losartan have been documented in the management of ventricular remodeling [111].

4.1.3. Losartan and Ventricular/Cardiomyocyte Hypertrophy—Cardiomyocyte hypertrophy and interstitial remodeling involved in fibroblast hyperplasia and transformation and extracellular matrix (ECM) remodeling can cause ventricular hypertrophy in coordination. Several clinical trials and experimental studies have demonstrated that losartan is extremely beneficial against these pathological processes [112,113].

Early studies showed that losartan inhibited Ang II-induced myocyte hypertrophy in neonatal and adult cardiomyocyte culture derived from the intact heart [114,115], and was benefit to pressure-independent ventricular hypertrophy resulting from Ang II infusion *in vivo* [116]. Further studies indicated that losartan played an anti-hypertrophic role in cardiac myocytes under various conditions that would lead to cardiac hypertrophy [117–119]. These conditions include volume overload [120], ischemia [121], and diabetes [122], which activated the local RAS in the heart. Losartan inhibits cardiomyocyte AT1 receptors in attenuation of hemodynamic overload (mechanical stress) owing to its hypotensive influence. Notably, the myocyte hypertrophy induced by a local elevation of cardiac Ang II may be caused *via* transforming growth factor- β 1 (TGF- β 1) against the direct activation of AT1 receptors by autocrine mechanisms [123]. The effects of losartan on cardiomyocyte hypertrophy are also attributed by blocking AT1 receptors on the fibroblast and myofibroblast cells in the cardiac interstitium *via* a paracrine mechanism [124,125]. Furthermore, losartan acting on fibroblasts decreases endothelin-1 and interleukin-6 (IL-6) that are secreted from fibroblasts to induce cardiomyocyte hypertrophy [126]. It is reasonable to speculate that losartan may break the AT1 receptor-mediated functional link between cardiomyocytes and fibroblasts for its anti-hypertrophic effects on cardiac myocytes. In addition, the detrimental effects of chronic and excessive enhanced β_1/α_2 -adrenergic action has been suggested as an additional mechanism serving in various cardiac injuries [127,128]. Thus, the blockade of AT1 receptors at the noradrenergic nerve terminals surrounding the myocardium should attenuate the sympathetic terminal drive, which probably is another mechanism in anti-hypertrophy by losartan.

4.1.4. Losartan and Cardiac Fibrosis—The cardiac interstitium, the space between the cardiomyocytes, is composed of fibroblasts, ECM, nerve terminals, interstitial neurons, and

blood/lymphatic vessels. Injury of the heart evokes a diverse and complicated array of responses related to the structural inappropriate rearrangement in the cardiac interstitium, which includes fibroblast proliferation and transformation, as well as fibrosis. The application of losartan is a widely accepted approach against such kind of pathophysiological changes.

In vitro studies demonstrated that losartan inhibited proliferation in the cultured cardiac fibroblasts triggered by Ang II in both neonatal and adult normal hearts [129]. *In vivo* studies showed that losartan attenuated proliferation of cardiac fibroblasts caused by a variety of diseases resulted in cardiac injury [130]. As discussed above, losartan inhibits the cross-linked signals between myocytes and fibroblasts in progression of cardiac hypertrophy.

Unlike cardiomyocytes, abnormally activated cardiac fibroblasts exhibit not only proliferation but also represent transformation, migration, and production of ECM proteins [131]. Cardiac fibrosis resulting from disproportionate increase in synthesis and inhibition of degradation of ECM proteins related to an over-activated RAS in diseased hearts [132], may be prevented by losartan [133]. A growing body of evidence showed that the elevated cardiac Ang II and AT1 receptors under a number of pathological conditions lead to an inappropriate progressive increase of ECM proteins, including fibronectin, collagen (particularly type I, III), and laminin, and integrin [128], as well as disturbance between matrix metalloproteinases (MMP) and tissue inhibitors of MMPs (TIMPs) [134,135]. Thus, losartan may have its anti-fibrosis role in diseased hearts *via* blocking AT1 receptors. However, it is noted that a number of other factors in paracrine and autocrine pathways, for instance, catecholamines, TGF- β 1, basic fibroblast growth factor (bFGF), and insulin-like growth factor-1 (IGF-1), also may be involved in fibrosis, which may act as direct effects in fibrosis at the downstream signaling of Ang II [136].

Taken together, the data achieved so far indicate that the protection effect on ventricular hypertrophy by losartan is not only simply due to its AT1 receptor blocker, but also *via* its targets in breaking down the successive cascade of harmful events.

4.1.5. Bypass Products of AT1 Blocker and Ventricular Hypertrophy—As mentioned above, AT2 receptors on both cardiomyocytes and fibroblasts are elevated in the diseased heart [137]. Furthermore, the receptor ratio of AT2/AT1 increases [138], especially in human [139]. Losartan inhibits the binding of Ang II to AT1 receptors. As a result, increased Ang II targets AT2 receptors. Growing evidence has suggested that the activation of AT2 receptors elicits opposing effects against the AT1 activation in hypertrophic hearts [140]. However, the exact influence mediated by AT2 receptors remains to be determined [141–145].

In addition, the ACE2 up-regulation induced by losartan may increase Ang [1–7] in the myocardium. Ang [1–7] has been suggested to play an anti-hypertrophic role [146] in the myocardium *via* its anti-collagen/fibronectin production, anti-trophic of fibroblasts [100], and anti-inflammation of prevasculature in the heart [147].

4.1.6. Losartan and Ventricular Electrical Remodeling—Changes in ventricular structures and functions promote the occurrence of various arrhythmias, termed as ventricular electrical remodeling. In ventricular hypertrophy, prolongation of the action potential is the most common electrophysiological alteration observed [148]. Evaluation of losartan in the elderly showed that losartan reduced the QT dispersion in elderly patients with heart failure [149]. Prolonged treatment with losartan is able to prevent action potential prolongation as a result of normalization of I_{to1} channel (transient outward potassium

channel) expression so as to normalize the duration of repolarization [150]. I_f (pacemaker current) channels in pacemaker cells are expressed in ventricular myocytes during fetal and neonatal phase but absent in adult ventricular myocytes. However, their re-expression is observed in the hypertrophied ventricular myocytes [151]. Interestingly, losartan down regulated the amplitude of I_f [150]. This may be one of the possible mechanisms by which losartan prevents the sudden death in elderly heart failure patients [152]. In addition, losartan also may prevent the sudden cardiac death due to its prevention of cardiomyocyte apoptosis [153] (Fig. 4). However, information on this issue is still limited so that more studies are required. Previous studies demonstrated that losartan and its metabolite —E3174 modified several delayed potassium currents (I_{Kur} , I_{Kr} , I_{Ks}) in an Ang II-independent manner [154]. However, the intrinsic interaction between losartan and potassium ion channels and the exact roles in ventricular arrhythmias are not clear.

As mentioned above, losartan elicits long-term Ang II-dependent effects on some cardiac ion channels, which may affect the electrical conductance in the heart. However, the sufficient evidence still lacks in judging exact anti-arrhythmic effects of losartan in its clinical use. Thus, losartan has not been included in the anti-arrhythmic drugs to date, although its beneficial actions in preventing some kinds of lethal arrhythmias (e.g. long QT interval, ventricular fibrillation) have been well noted. Losartan's protective effects against atrial fibrillation (AF) are discussed below.

4.1.7. Alteration of Motor Molecular Phenotype by Losartan—Under the condition of ventricular remodeling, various fetal molecules in “reprogramming” emerge in cardiac myocytes related to β -MHL (myosin heavy chain), skeletal α -actin, and ANF (atrial natriuretic factor) [155]. With application of losartan, mRNA of these molecules was decreased or normalized in a consistent way in a variety of experimental models [155]. It is well accepted that the β -MHL, which has low ATPase activity, is predominantly expressed in the fetus. In contrast, the intact adult cardiomyocyte has α -MHL in majority, whose ATPase is high. Similarly, the skeletal α -actin owing greater contractility is in fetal and neonatal form, whereas normal adult predominantly possesses the cardiac α -actin with less contractility. Losartan may alter these molecules and their performance *via* its reversible effects on the molecular “reprogramming”.

4.2. Losartan and the Atrium

4.2.1. Losartan and Atrial Remodeling—Although ventricular and atrial remodeling cannot be separated exactly, it is relatively simple for understanding these issues by discussing them separately. Not fully like what we know about the ventricular remodeling [156], the atrial fibrosis, especially the atrial electrical remodeling, is the most important phenotype in the genesis of atrial remodeling. In addition, atrial enlargement also has been paid attention.

4.2.2. Losartan and Atrial Structural Remodeling—Atrial dilation and interstitial fibrosis, the main structural changes of the atrium, are mediated by the activation of the cardiac local RAS [157,158]. Losartan abolishes the atrial enlargement and fibrosis *via* its direct blocking effects on AT1 receptors [159,160]. It is of importance that myocytes occupy about 45% (V/V) in the atrial myocardium, compared with approximately 76% in the ventricles [161,162]. The greater atrial interstitium could become exaggerated within remodeling processes. Consequently, the cardiomyocyte–fibroblast crosstalk described above also may be involved in promotion of atrial fibrosis. Thus, the fibroblast-ECM ought to be the key target of medical application of losartan.

4.2.3. Losartan and Atrial Fibrillation—Atrial fibrillation is generally thought to be the major outcome of electrical remodeling in the atrium followed alterations of the intracardiac RAS resulting from various cardiovascular diseases [163]. Ang II exquisitely elicits ionotropic effects on cardiomyocytes *via* its AT1 receptor activation. As mentioned above, Ang II may be increased under various medical conditions. The elevated Ang II in the atrial myocardium elicits an increase of L-type calcium influx (I_{CaL}), slows component of delayed rectifier potassium current (I_{Ks}), and shortens action potential duration (APD) [164,165]. Furthermore, increased Ang II also contributes to the disorganization of the intercalated discs (e.g. connexin 43 disturbances), increases the activity of sodium/calcium anti-transporter (NCX) and abnormal calcium leak from the sarcoplasmic reticulum [164,166]. Several consequences have been noted following an increase of local Ang II in the atria. First, an increased I_{Ks} and decreased APD shorten the effective refractory periods (ERP). Second, increased energy dissipation between current “source” and “sink” resulting from impaired functions of the gap junction (e.g. connexins disorders) and disturbed cable-like cardiomyocyte bundles due to atrial fibrosis slow the conduction in the atrium. Third, the enlargement of the left atrial chamber, whose dimension can accommodate the long-wavelength circuits that are too large for a normal atrium to support, usually occurs following chronically activation of the cardiac RAS. Subsequently, the foundation for the maintenance of reentry develops. In addition, the calcium overload (increased I_{CaL}) and exaggerated calcium leak induced by chronically elevated Ang II cause the formation of ectopy in the atrium (pulmonary veins sleeved by left atrium should be more prone to be a trigger). Consequently, a persisted increase of intracardiac Ang II may potentially induce and maintain AF independently [167]. In light of medical prevention, recent evidence has shown that losartan may abolish AF with the pleiotropic mechanisms of the direct abrogation of ionotropic effects of Ang II [159].

The attenuation of interaction between Ang II and catecholamines by losartan also plays an important role [168] in the protection of the heart. For example, the decreased noradrenalin resulting from the AT1 receptor blockade on the prejunction loci of the sympathetic nerve endings innervating the myocardium, may be beneficial to exited automaticity in the heart. Furthermore, an elevated activation of AT2 receptors in part inhibits the L-type calcium channel activity *via* its transduced phosphatases activity, and in turn, may be an additional effect of losartan on electrical activity of atrium [164].

4.3. Losartan and Myocardium Inflammation

In some kinds of diseased hearts, especially pressure overload and injury induced cardiac problems, inflammatory process may occur. Certain inflammatory cells, identified mainly as macrophages and T lymphocytes [169,170], infiltrate the perivascular domain, and subsequently, the interstitial space in the myocardium. The activation of AT1 receptors localized on these inflammatory cells [171,172] may increase adhesion of monocytic cells to the endothelium [173,174]. As a result, application of losartan attenuates myocardial inflammation by the inhibition of proinflammatory role of AT1 receptor activation [175]. Furthermore, losartan may block the subsequent interaction among inflammation, fibrosis, and cell death [176].

On the other hand, chymase synthesized and secreted from inflammatory cells and mast cells, as well as cathepsin G released from neutrophils, are alternative enzymes for the activation of the intracardiac RAS [177,178]. As a result, application of losartan may lower the cardiac Ang II *via* its antagonistic pathway.

4.4. Losartan and Cardiac Cell Apoptosis

Cardiomyocyte apoptosis takes part in diseased hearts [179]. The anti-apoptotic effect of losartan influences both cardiomyocytes and fibroblasts *in vitro* [180–182]. Chronic treatment with losartan *in vivo* reduces cardiomyocyte apoptosis by inhibiting AT1 receptor activation [183,184].

Although Ang II has been shown to induce apoptosis in other cardiovascular cells *via* stimulation of AT2 receptors [185], it is suggested to be unlikely that this type of receptors is a potent activator to induce apoptosis of cardiomyocyte *in vivo* [186].

4.5. Integrated Benefits of Losartan to Cardiac Functions

The benefits of losartan to diseased hearts are integrated, which can be summarized as below:

1. **Systolic functions:** Losartan prevents and reverses the “fetal reprogramming” of the contractile molecules, which strengthens and fastens the cardiomyocyte contractility, as well as elevates the efficiency of heart energy utilization. The roles of anti-perivascular inflammation/fibrosis, dilation of the coronary vasculature, and anti-interstitial remodeling further improve blood supply in order to restore effective energy resources for contraction of the cardiomyocyte. The anti-apoptosis effect helps the stability of the “cardiomyocyte repertoire”. Proper dilation provides a correct volume of the end phase of dilation, which represents a proper ejection ratio. Properly organized electrical impulses raise a well coordination between the atrium and ventricle, and an effective phase-2 plateau to elicit an effective contraction.
2. **Diastolic functions:** The anti-remodeling effect of losartan effectively contributes to the modification of “stiffness” of the heart. The morphology and functions of the recovered cardiomyocyte favor adequate dilation of the myocardium. A well regulated systolic function gives rise to a properly controlled volume of the end phase of systole, which is beneficial to formation of a good negative pressure for blood return to the atrium. The regressed electrophysiological properties effectively support a good phase-3 repolarization for a proper dilation.
3. **Electrophysiological functions:** The potential anti-arrhythmia roles in the atrium and ventricle by chronically application of losartan maintain an ordered automaticity, conduction, and electrophysiological activity, which in turn is very important to cardiac functions.

5. LOSARTAN AND VASCULAR RAS ACTIVATION

5.1. Losartan and Vascular Tone

The vascular tone is undoubtedly maintained and regulated by both smooth muscle cells (SMC) and the endothelium. Several lines of evidence showed that losartan could influence vascular tone targeting either SMC or the endothelium [155].

5.1.1. Losartan and SMC Contractility—Ang II causes contractions of SMC resulting from an elevation of intracellular calcium and enhanced protein kinase C (PKC) activity by activation of phospholipase C mediated *via* G_q-coupled AT1 receptors [187]. Losartan decreases vasoconstriction *via* its direct abrogation of AT1 receptor activation in SMC. In addition, the emerged AT2 receptor in SMC is subsequently activated after the blockade of AT1 receptors by losartan, which may elicit an additional vasodilatation mediated by increased phosphatase activity in SMC [188]. Furthermore, several alternative

vasoconstrictive factors, including endothelin-1, may be augmented by activation of AT1 receptors [189]. Consequently, losartan may indirectly reduce vascular contractility.

5.1.2. Losartan and Endothelial Functions—With the use of losartan, the increased Ang II from blocking of AT1 receptors in turn bind to AT2 receptors located on the endothelial cells. Elevated activity of NO-cGMP and endothelial hyperpolarization factors subsequent to AT2 receptor activation in the endothelium induce vascular dilation [70]. Moreover, subsequently increased Ang [1–7] may also result in vasodilation *via* the Mas receptor pathway [67], which contributes to the effects of counter-constriction of losartan. Thus, losartan effectively decreases SMC constriction and increases endothelium-dependent dilation simultaneously and reciprocally. Furthermore, the attenuation of ROS owing to AT1 receptor blocking further strengthens the function of NO.

5.2. Losartan and Inflammation in the Vasculature and Vascular Permeability

Growing evidence shows that increased Ang II induces vascular inflammation either dependent or independent of its hemodynamic effects [190,191]. Once inflammation is triggered, vasculature changes may undergo: a) increased vascular permeability; b) leukocyte adhesion and chemotaxis; and c) vascular repair [192], as discussed below (Fig. 5).

5.2.1. Losartan and Increased Vascular Permeability—Elevated Ang II results in a damage of filter barrier of vascular walls [193]. Prostaglandins (PGs) have been long considered to be important mediators involved in the regulation of vascular permeability [194]. A number of studies demonstrated that increased Ang II stimulated synthesis and secretion of several PGs, including LTC₄ (leukotriene C₄), PGE₂, and PGI₂, from either endothelial cells [195] or SMC [196], which was abrogated by losartan [197]. Moreover, both clinical and experimental data indicated that the activated RAS was able to induce expression and secretion of vascular endothelial growth factors (VEGF) in the endothelium and vascular SMC [198], trigger angiogenesis, and increase vascular permeability [199,200], which were primarily mediated by the activation of AT1 receptors [201]. Therefore, losartan may be used against impairment of vascular permeability *via* the mechanism of preventing the generation of PGs and VEGF by its depressor effects [202]. In addition, as high blood pressure leads to endothelial injury [203], losartan may display beneficial effects on vascular permeability by its anti-mechanical insults [204].

5.2.2. Losartan and Leukocyte Infiltration and Recruitment—Leukocytes infiltration (including margination and transmigration) from vascular lumen into interstitial space is a crucial phase in inflammatory responses. Recent data indicated that Ang II increased the expression of E-selectin and P-selectin in the endothelium [205,206] *via* either AT1 or AT2 receptor pathways and prompted the elevation of L-selectin in leukocytes [207], provoking a weak adhesion interaction between endothelial cells and leukocytes (i.e. rolling) [208]. Intercellular adhesion molecules-1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) expressed in endothelial cells and vascular SMC [209–211], and $\alpha_4\beta_2$ -integrin located in leukocytes, are generally considered to be involved in adhesion step of margination. They also may be directly enhanced by AT1 receptor activation. In addition, previous studies demonstrated that increased Ang II, predominantly mediated by AT1 receptor independent of its pressor effects, participated in synthesis of CXC chemokines (e.g. MIP-2 (macrophage inflammatory protein-2), interleukin-8 (IL-8), cytokine-inducible neutrophil chemoattractant/keratinocyte-derived chemokine (CINC/KC) [211–213], CC chemokines (e.g. monocyte chemoattractant protein [MCP-1]), expressed and secreted by normal T cells (RANTES) [214,215] from endothelial cells, SMC, and monocytes/macrophages in the vasculature, which in turn stimulated transmigration of leukocytes

towards the chemoattractant gradients to recruit in the interstitial spaces [216]. Inflammatory cells are equipped with almost all of the RAS components, and in turn to produce intrinsic Ang II [217], which may further activate and up-regulate phagocytic and chemotactic activity of the monocytes/macrophages [218]. This also indicates that losartan prevents not only acute, but also chronic activation of leukocytes in the vascular interstitium. Taken together, losartan may effectively attenuate leukocyte infiltration by blocking, at least in part, both margination and transmigration phases, and inhibit recruitment [212,217].

Moreover, recent data have indicated that the homing and infiltration of double-negative T cell (CD3⁺CD4[−]CD8[−]) expressing CC chemokine receptor 5 (CCR5) is mediated by AT1 receptor activation [219]. In addition, the increased plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, which acted as an acute phase reactant in various cardiovascular diseases, is lowered by application of ARB [220]. These works provide alternative targets for losartan, although further studies are needed.

5.2.3. Losartan and Vascular Repair—Subsequent to acute or sustained inflammatory responses, damaged vasculature may undergo recovery through processes related to cell growth and ECM remodeling as discussed below.

5.3. Losartan and Vascular Remodeling

Vascular remodeling can be divided into hypertrophic remodeling [predominantly occurs in large blood vessels characterized by an increase in both media-to-lumen ratio and medial cross-sectional area [221]] and eutrophic remodeling [i.e. increased media-to-lumen ratio together with constant in medial cross-sectional area, which is common in resistance vessels [222]]. Vascular remodeling is induced by various cardiovascular diseases, especially by hypertension [223]. Growing evidence shows that chronically activated the vascular RAS is involved in the pathogenesis of vascular remodeling [224]. Recent studies have indicated that losartan effectively affects vascular remodeling [225].

5.3.1. Losartan and Large Blood Vessel Remodeling—Increased vascular Ang II stimulates hypertrophy and proliferation of SMC through AT1 receptors both *in vitro* and *in vivo* [226]. Increased levels of Ang II in the vasculature are involved in fibrosis. Over activation of AT1 receptors results in fibronectin, collagen, and laminin synthesis and secretion from SMC [226], which may be secondary action by the induction of elevated TGF- β_1 (similar as seen in the heart) and CTGF [227–229]. Furthermore, elevated Ang II causes SMC apoptosis and abnormal ECM deposition *via* AT1 receptors by enhancing TIMP and attenuating MMP activity [230]. Due to competitive inhibition to AT1 receptors, losartan effectively, at least in part, inhibits the SMC injury (i.e. hypertrophy, hyperplasia, apoptosis, and ECM deposition) [231]. It was noted that recruitment of the inflammatory cells in the interstitium of the vessel wall resulted in vascular remodeling *via* a complex network, which had been extensively reviewed previously [232]. Certainly, the attenuation of losartan to inflammation as discussed above may be able to prevent the occurrence of consequent repair responses in the vascular tissue. Moreover, losartan may inhibit at least in part the secretion of chymase from activated and recruited mast cells in the intima, *via* its blockade effects on AT1 receptors locating on mast cells [233]. In addition, the enhanced AT2 receptor activation in SMC also plays a role in the anti-fibrotic effect of losartan [234].

5.3.2. Losartan and Resistance Vessel Remodeling—The reorientation of smooth muscle cells around a smaller lumen may be a result of chronic contraction by sustained activation of AT1 receptors in SMC, possibly through the ERK transduction pathway, in the resistance vessels [223,235]. In the development of intrinsic cardiovascular diseases and severe hypertension, vascular remodeling at tissue, cellular, and molecular levels in

resistance vessels is consistent with that in large conductance vessels described above. The amelioration effect of losartan in this pathological process has been demonstrated [236].

5.3.3. Losartan and Atherosclerosis—A variety of cardiovascular diseases, especially complicated with disorders of lipid metabolism, lead to the occurrence and development of atherosclerosis. Coronary atherosclerosis is the most common and severe one in clinic. Recent studies have shown that losartan effectively ameliorates atherosclerotic diseases [237,238].

The intact endothelium is considered to be the first place of protection against atherosclerosis [239]. As discussed above, losartan ameliorates endothelial functions, and a subsequent increase of NO and decrease of ROS are further beneficial in the prevention of initiation of inflammation and thrombosis [232,240].

Inflammation has been widely accepted to be a potential mechanism underlying the pathogenesis of atherosclerosis [232]. The anti-inflammatory role elicited by losartan also may be amelioration to this pathological process. Furthermore, the inflammatory factors (e.g. VCAM, IL-6, IL-10, etc) are indeed decreased by the application of ARB [241–243]. Plaque rupture and subsequent thrombosis are considered to be a natural phase in coronary atherosclerosis. It has been demonstrated that losartan prevents the increase of PAI-1 induced by Ang II [220,244,245], and plays an important role in the prevention of coronary atherosclerosis.

6. CONCLUDING REMARKS

An abnormal or over activation of local tissue RAS in the heart and the vasculature plays a crucial role in pathological changes in the cardiovascular tissue such as cardiac remodeling and vasculature inflammation. The AT1 receptor antagonist losartan is one of good candidates in prevention and treatments of such kind of pathological process in cardiovascular diseases. From the cellular and molecular mechanisms discussed in this review, *via* which an over-activated local RAS in the cardiovascular system, and sometimes accompanied with the systemic RAS, exerts its actions by AT1 receptors, in producing cardiac / vascular remodeling, ventricular / cardiomyocyte hypertrophy, cardiac electrical reforming, atrial fibrillation, myocardium / vascular inflammation, and atherosclerosis. Understanding the chemical pathway(s) in those cellular and molecular mechanisms will assist us not only in medicational use of existing Ang II receptor antagonists such as losartan, but also in development of potential new compounds in medical prevention and treatments against various cardiovascular diseases. In reviewing of chemistry of the AT1 receptor, and interactions between the AT1 receptor and losartan, as well as the effects of losartan on the heart and vasculature, this article provides interesting information on the insightful mechanisms underlying actions of losartan in the heart and vasculature. This may be helpful in development of new chemical candidates or approaches and in medical applications of losartan in the war against the cardiovascular diseases that cost human health and life.

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Fig. (1). The sequence of the human AT1 receptor

The seven transmembrane helices are depicted in the standard 4-3-4-3 format as α helix, which are modeled according to the resolved rhodopsin structure. The three N-glycosylated sites (Asn⁴, Asn¹⁷⁶, and Asn¹⁸⁸) are highlighted. The two sulfide bonds detected between Cys¹⁸ and Cys²⁷⁵, Cys¹⁰¹ and Cys¹⁸⁰, respectively, which have been demonstrated to maintain three dimensional conformation of the receptor protein. The possible interaction residues between AT1 receptor and losartan are involved in six sites (Thr¹⁹⁸, Ser²⁵³, Asn²⁹⁴, Asn²⁹⁵, Leu³⁰⁰, and Phe³⁰¹). An S-palmitoyl-cysteine in Cys³⁵⁵ is at the end of C-terminal tail. The eighth α helix in the cytoplasm in the receptor is derived from the crystal structure of rhodopsin.

Fig. (2). The ribbon model of tertiary structure of AT1 receptors and possible interaction between losartan and AT1 receptors

Left panel shows the ribbon model of AT1 receptor derived from resolved crystal structure of rhodopsin. The seven transmembrane helices (I–VII helix) are highlighted in blue, dark green, bright green, orange, yellow, cyan, and red, respectively. The eighth cytoplasmic α helix (helix VIII) following the helix VII is in red, horizontally, which shares an about 90° angle with the seventh helix. The black lines indicate the loops connecting successive helices. The possible interaction between losartan and AT1 receptors is shown in right panel. Losartan is presented in yellow barrel, indicating hollow space in the seven-transmembrane bundle buried in the bilipid membrane. Once losartan interact with residues in the extracellular halves in the helix V and VI (Thr¹⁹⁸, Ser²⁵³), it can extend through cavity in the seven-transmembrane bundle among helix III, VI, and VII. Subsequently, losartan interacts with residues in the cytoplasmic halves of helix III (Asn111), and helix VII (Asn²⁹⁴, Asn²⁹⁵), as well as residues in the interface between membrane and cytoplasm (Leu³⁰⁰, Phe³⁰¹).

Fig. (3). Losartan and cardiac structural remodeling

AT1 receptors are detected in both cardiomyocytes and fibroblasts in the myocardium. Sustained elevation of Ang II triggers and increases transcriptional activity of TGF-β1 in either cardiomyocytes or fibroblasts mediated by AT1 receptors. Subsequently, the increased neosynthesized TGF-β1 can be excreted into the cardiac interstitial space, which in turn stimulates TGF-β1 receptors in the surrounding cardiomyocytes and fibroblasts by autocrine or paracrine route. Over activation of TGF-β1 receptors elicits various downstream effects, including cardiomyocyte hypertrophy, cell growth and/or transformation of fibroblast, synthesis of ECM components (such as collagen, laminin, fibronectin). In addition, activation of AT1 receptors locating on the sympathetic nerve terminal innervating the heart can lead to overload of catecholamine in the cardiac tissue. As a result, activation of β1 receptors in cardiomyocytes also can induce cardiac remodeling. Losartan can, at least in part, inhibit AT1 receptors to abolish detrimental outcomes originating from the initial step.

Fig. (4). Anti-arrhythmia effects of losartan

Elevated and sustained activation of AT1 receptors in cardiomyocytes, especially in the ventricle, could reprogram activation and increase of I_f channel (on target HCN2 gene in ventricular myocytes) expression, as well as inhibition of expression of I_{to} channel (predominantly on Kv4.2 and Kv4.3 gene). The overt emergence and increased I_f current, associated with abnormal action potential duration (APD) resulting from decreased I_f current in the ventricle, can trigger an ectopic stimulus and dispersion of QT interval, which would then induce several lethal ventricular arrhythmias, for instance, acquired long QT syndrome (LQTS), Torsade de Pointes (TdP), and ventricular fibrillation. On other hand, losartan blocks abnormal electrical remodeling *via* inhibition of AT1 receptors. In addition, losartan and/or EXP3174 (the oxidation metabolite of losartan) can directly inhibit delayed potassium currents (including ultra-rapid delayed potassium current, I_{Kur} , rapid delayed potassium current, I_{Kr} , and slow delayed potassium current, I_{Ks}), so as to shorten the APD to avoid development and occurrence of cardiac sudden death. Losartan also can ameliorate atrial fibrillation (AF). The persisted stimulation from increased Ang II can, *via* AT1 receptors in atrial myocytes, affect L-type calcium current (I_{CaL}) and inhibit I_{Ks} . Furthermore, calcium leak induced by disordered calcium communication between the sarcoplasmic reticulum and cytoplasm may be deteriorated by abnormal activation of AT1 receptors, associated with the augmentation of calcium influx owing to activation of I_{CaL} current that would consequently over-stimulate the NCX (sodium-calcium exchanger). Taken together, ectopy in the atrium would accessibly develop and progress. In addition, expression of connexins inhibited by AT1 receptor activation can result in failure of gap junction assembly, and then to slow conduction velocity in the atrium. Integrating electrical anomalies and atrial geometric changes (atrial enlargement induced by AT1 stimulation), the AF would develop. Losartan can abrogate all those events elicited by AT1 receptor activation.

Fig. (5). Losartan and vasculature inflammation and remodeling

Sustained elevation of AT1 receptor activation in endothelial cells and vascular smooth muscle cells (SMC) would increase synthesis and secretion of both prostaglandins (PGs, e.g. LTC₄, PGE₂, and PGI₂) and vascular endothelial growth factor (VEGF), then increase vascular permeability and trigger inflammation. Increased expression of E-, P-selectin in endothelial cells and SMC, and L-selectin in leukocytes, caused by AT1 receptor activation, could induce initial step of inflammation. Moreover, owing to sustained activation of AT1 receptors, intercellular adhesion molecules-1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) can be elevated in both endothelium and SMC, and integrins (particular $\alpha 4 \beta 2$ -integrin) located in leukocytes also can be increased. As a result, adhesion phase of margination can be developed. Vascular interstitial chemokines (including CXC chemokines, e.g. MIP-2, IL-8, CINC/KC; CC chemokines, e.g. MCP-1, RANTES), being culprits to transmigration of adhered leukocytes, can be increased due to elevated synthesis and secretion from the endothelium, SMC, and leukocytes resulting from persistent stimulation of AT1 receptors in these cells. Consequently, chronic activated and recruited leukocytes, and AT1 stimulated SMC could in turn synthesize and secrete various extracellular matrix (ECM) components (e.g. fibronectin, laminin, collagen), and induce the MMP/TIMP disturbance in the interstitium. Furthermore, sustained AT1 receptor activation in SMC elicits injury in SMC (i.e. hypertrophy, hyperplasia, and apoptosis). And the recruited leukocytes and activated mast cells in the interstitium also can induce SMC injury. This figure showed the blockade ways for those events by losartan. Losartan also can partially attenuate chymase secreted from mast cells by inhibition of AT1 receptors.